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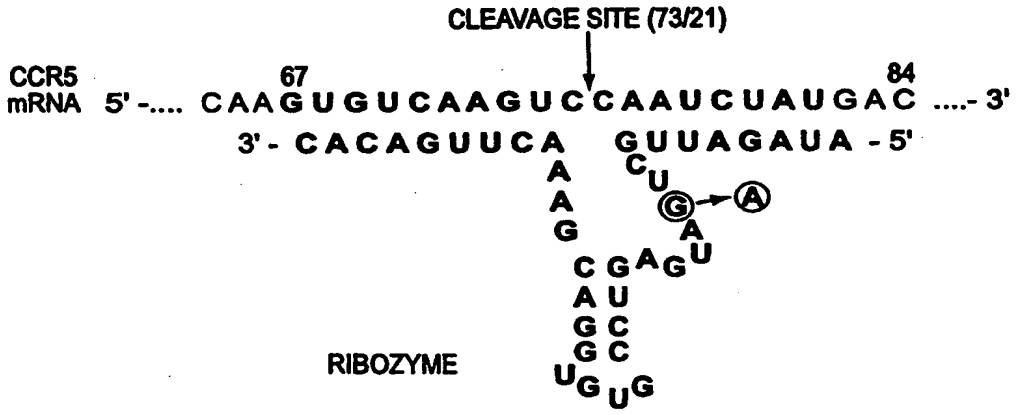

(57) Abstract

This invention provides ribozymes and combinations thereof, to cleave RNA sequences. The invention also provides a method of treating HIV–1 by down–regulating the CCR5 receptor.

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RIBOZYMES CAPABLE OF INHIBITING THE EXPRESSION OF THE CCR5 RECEPTOR

GOVERNMENT RIGHTS STATEMENT

This invention was made with government support under Grant No. AI 29329 awarded by the National Institutes of Health. The government has certain rights in the invention.

Field of Invention

This invention relates to ribozymes and combinations thereof. More particularly, the invention broadly involves regulation of CCR5.

BACKGROUND OF THE INVENTION

The concept of genetic therapies for providing intracellular immunity to viral infection have been entertained for a number of years (see Baltimore, 1988; Szydalski, 1992). Gene therapy has recently received more attention for its potential utility in the treatment of HIV infection (Sarver and Rossi, 1993). A number of different inhibitory agents have been tested for their ability to confer resistance to HIV-1, including anti-sense RNA, ribozymes, TAR or RRE decoys, trans-dominant mutant HIV genes and conditionally lethal toxins (reviewed in Sarver and Rossi, 1993).

RNA-based strategies, such as anti-sense or ribozymes, have the dual advantage of being sequence specific, theoretically eliminating unwanted

toxicities, as well as not producing potentially immunogenic proteins. A single ribozyme molecule is capable of irreversibly inactivating multiple target RNA molecules by sequential cycles of binding, cleavage and release. Even in the absence of multiple substrate turnover, ribozymes functionally inactivate target RNAs via cleavage (Zaug and Cech, 1986; Uhlenbeck, 1987; Castanotto et al., 1992).

Recently it has been discovered that individuals harboring a 32-base homozygous deletion in the CCKR-5 (also known as CCR5) gene are not subject to an infection by an M-tropic HIV-1 strain. Moreover, heterozygotes are long term survivors, which suggests that a defect in the CCR5 expression may interfere with the normal progression of AIDS. The protein encoded by the 32-based deletion gene is severely truncated, undetectable at the cell surface and with no obvious phenotype in homozygous individuals. This suggests that the inhibition of the CCR5 expression at the cell surface should affect the HIV-1 entry.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a schematic diagram indicating the U6 promoter construct used to transcribe an anti-CCR5 ribozyme.

Figure 2 illustrates the computer predicted secondary structure of VA1 (A) and VA1-anti-CCR5 ribozyme (B).

Figure 3 is an in vitro ribozyme cleavage reaction comparing U6+19-CCR5 ribozyme and U6+27-CCR5 ribozyme.

Figure 4 is an in vitro ribozyme cleavage reaction using VA CCR5rz and VACCR5rzm with the CCR5 substrate.

Figure 5 illustrates an anti-CCR5 hammerhead ribozyme and target sequence.

Figure 6 depicts detection of VA1 and VA1-CCR5rz RNA in transiently transfected cells.

Figure 7 is a northern blot from 293 cells transfected with pVA1 (lane 1), pVA1 anti-CCR5 ribozyme (lanes 2 and 3), and pVA1 anti-CCR5 ribozyme mutant (lanes 4 and 5).

Figure 8 illustrates a RNA polymerase II expression system.

Figure 9 illustrates a RNA polymerase III expession system.

Figure 10 is a graph depicting down regulation of CCR5 receptor in cell culture.

SUMMARY OF THE INVENTION

This invention provides a method of treating HIV infection by down regulating CCR5 in mammalian cells. In other aspects, the invention provides novel ribozymes targeted against the CCR5 HIV-1 co-receptor.

The invention also provides a method of making HIV-resistant cells with vectors that express anti-CCR5 ribozymes. In preferred embodiments, the anti-CCR5 ribozyme is used in combination with one or more ribozymes targeted to conserved sequences in HIV.

DETAILED DESCRIPTION OF THE INVENTION

CCR5 is a seven transmembrane receptor for the beta-chemokines, MIP1-alpha, MIP1-beta and RANTES. Several studies have demonstrated the ability of these chemokines to inhibit HIV-1 infection of CD4+-T lymphocytes and to inhibit syncytia formation in HIV-infected cells. Individuals harboring a 32-base homozygous deletion in the CCR5 gene are not subject to

infection by a M-tropic HIV-1 strain. Moreover, heterozygotes are long-term survivors, which raises the possibility that a defect in the CCKR-5 expression may interfere with the normal progression of AIDS. The protein encoded by the 32-based deletion gene is severely truncated, undetectable at the cell surface and with no obvious phenotype in homozygote individuals. Thus, it was hoped that the inhibition of the CCR5 expression at the cell surface would affect the HIV-1 entry, making downregulating CCR5 expression an attractive therapeutic approach for prevention and treatment of HIV-1 infection.

The present invention provides, among other features, a novel approach for downregulating CCR5 with ribozymes. The invention provides, in its various aspects, methods and compositions for altering the expression of the CCR5 receptor. Combinatorial vectors that express anti-CCR5 ribozymes, optimally in combination with one or more ribozymes targeted to conserved sequences in HIV, are used to transduce CD34+ human hematopoietic precursor cells, which in turn will give rise to HIV resistant mononuclear cells.

A number of classes of catalytic RNAs (ribozymes) have been described in the literature, and the present invention is not limited to any one class of ribozyme. In a preferred aspect, however, the ribozymes of the present invention are "hammerhead" ribozymes. Such ribozymes have a hybridizing region (conferring the desired specificity) comprising one or more arms formed of single-stranded RNA having a sequence complementary to at least part of a target nucleic acid, such as mRNA. The hybridizing (or "anti-sense") regions

comprise segments of RNA typically containing a sufficient number of nucleotides to effect hybridization to the target nucleic acid. Typically, these regions will contain at least about seven nucleotides, preferably from about nine to about twelve nucleotides. A conserved catalytic core region is capable of cleaving the targeted RNA. The preferred ribozymes of the present invention cleave target RNA which contain the sequence X_1UX_2 where X_2 is adenine, cytosine or uracil and U is uracil. Preferably, X_1 is guanidine, and X_1UX_2 is GUC or GUA.

The anti-sense arms of the ribozymes can be synthesized to be complementary to, and thus hybridizable to the RNA on the target CCR5 mRNA sequence flanking the chosen X_1UX_2 sequence. Upon hybridization of the anti-sense regions of the ribozyme to the target RNA sequence flanking the X_1UX_2 sequence, the catalytic region of the ribozyme cleaves the target RNA within the X_1UX_2 sequence. RNA cleavage is facilitated in vitro in the presence of magnesium or another divalent cation at a pH of approximately 7.5.

In one embodiment of the invention, there is provided a hammerhead ribozyme as illustrated in Figure 2. This ribozyme comprises a catalytic region having the sequence 3'-CACAGUUCAAAGCAGGUGUGCCUGAGUAGUCGUUAGAUA-5' (SEQ ID NO. 1) that recognizes a GUC sequence which is positioned immediately downstream of the CCR5 AUG initiation code. Specifically, the ribozyme targets against the second GUC of the CCR5 mRNA, from nucleotides 67 to 84 of the gene. The sequence in this region of the CCR5 mRNA is: 5'-GUGUCAAGUCCAAUCUAU-3'

(SEQ ID NO. 2). Cleavage occurs after the C of the second GUC triplet (Fig. 5). The ribozyme interacts with its target by two short arms of 9 and 8 nucleotides each. To insure that this ribozyme does not target other members of the chemokine receptor family or other endogenous transcripts, the exact sequences from CCR5 which base pair with the ribozyme were entered in a BLASTN search of Genbank and no significant homology was found with any other essential gene.

Those skilled in the art will appreciate that the sequence of the ribozyme of Figure 2 can be modified without departing from the invention. The catalytic region can be targeted to any X_1UX_2 sequence within the CCR5 mRNA, with the proviso that the X_1UX_2 sequence should be selected so as to result in the cleavage of the mRNA into one or more RNA strands that are incapable of serving as templates for the translation of a functional CCR5 molecule. Anti-sense regions capable of effectively bonding to bases (preferably 7-12 bases) upstream and downstream from the selected X_1UX_2 sequence will be selected based upon knowledge of the mRNA sequence.

The ribozymes can be further modified to include nuclease-resistant RNA bases. These modifications include, for example, the use of phosphorothioate derivatives of nucleotides (reviewed in Bratty et al., Biochem, Biophys. Acta 1216: 345-359 (1993)) To confer resistance to nucleases which degrade the ribozyme. The phosphorothioate group is introduced into the oligonucleotide using RNA or DNA polymerase and the corresponding nucleotide alpha-thiotriphosphate.

Alternatively, the phosphorothioate group is inserted at specific positions and in oligomer as a phosphoramidite during chemical synthesis.

The ribozyme also can be synthesized in the form of a chimeric ribozyme containing deoxyribonucleotide as well as ribonucleotide bases. These chimeric ribozymes have been shown to have increased cellular stability while maintaining efficient cleavage properties. The chemistry of chimeric (DNA-containing) ribozymes (also known as "nucleozymes") is reviewed in Bratty et al. supra. For original article, see Taylor et al., Nucleic Acids RES., 20: 4559-4565 (1992).

Inasmuch as ribozymes act intracellularly the uptake of ribozymes by the targeted cells is an important consideration and advantageously is optimized. A preferred method for exogenous administration of a ribozyme is through the use of liposomes. Liposomes protect the ribozyme against enzymatic attack and the liquid capsule of the liposome facilitates transfer through the cell wall. Liposomes have been developed for delivery of nucleic acids to cells. See, e.g., Friedmann, Science, 244:1275-1281 (1989).

Direct cellular uptake of oligonucleotides (whether they are composed of DNA or RNA or both) per se presently is considered a less preferred method of delivery because, in the case of ribozymes and anti-sense molecules, direct administration of oligonucleotides carries with it the concomitant problem of attack and digestion by cellular nucleases, such as the RNAses. One preferred mode of administration of anti-CCR5 ribozymes takes advantage

of known vectors to facilitate the delivery of a gene coding for the desired ribozyme sequence such that it will be expressed by the desired target cells. Such vectors include plasmids and viruses (such as adenoviruses, retroviruses, and adeno-associated viruses) [and liposomes] and modifications therein (e.g., polylysine-modified adenoviruses [Gao et al., Human Gene Therapy, 4:17-24 (1993)], cationic liposomes [Zhu et al., Science, 261:209-211 (1993)] and modified adeno-associated virus plasmids encased in liposomes [Phillip et al., Mol. Cell. Biol., 14:2411-2418 (1994)]. Expression of ribozyme RNA is driven by genetic elements such as RNA polymerase II and III.

The ribozymes of the present invention may be prepared by methods known in the art for the synthesis of RNA molecules. In particular, the ribozymes of the invention may be prepared from a corresponding DNA sequence (DNA which on transcription yields a ribozyme, and which may be synthesized according to methods know per se in the art for the synthesis of DNA) operably linked to a promoter. The DNA sequence corresponding to a ribozyme of the present invention may be ligated into a DNA transfer vector, such as a plasmid, bacteriophage DNA or viral DNA. Procaryotic or eukaryotic cells (including mammalian implanted cells) may then be transfected with an appropriate transfer vector containing genetic material corresponding to the ribozyme in accordance with the present invention, operably linked to a promoter, such that the ribozyme is transcribed in the host cell. Ribozymes may be directly transcribed from a transfer vector, or, alternatively, may be transcribed as part of a larger

RNA molecule which then may be cleaved to produce the desired ribozyme molecule. While various methods of transforming cells so as to produce the desired ribozyme are described herein, those skilled in the general field of non-native (recombinant) gene expression in mammalian cells will apply known techniques to provide additional means and methods for providing or optimizing ribozyme expression in CCR5 producing cells.

The ribozyme encoding sequence of Figure 5 has been chemically synthesized and cloned into four different expression vectors. The first two vectors are derived from the human U6 gene described in Bertrand et al. 1997 and Good et al., 1997 (Fig. 1). This is a Pol III cassette in which the promoter is 5' to the transcribed sequences. The difference between the two constructs resides in the amount of U6 sequence included in the RNA transcripts. The first 19 bases of this RNA form a stabilizing stem-loop (Bertrand et al., 1997), but lack information for capping (Fig. 8). The additional eight bases included in the U6+27 result in capping of the RNA with a gamma methyl phosphate (Singh, Gupta and Reddy, 1990; Goode et al., 1997) (Fig. 9). The U6+19, although primarily nuclear, can also be found in the cytoplasma to varying degrees (Bertrand et al., 1997), whereas the U6+27 sequence is exclusively nuclear. Advantageously, a stabilizing 3' stem-loop structure that is transcribed in both of these promoter cassettes may be appended to the ribozyme sequence. In order to evaluate the relative cleavage activities of the ribozymes with the appended 5' and 3' sequences, RNAs from both the U6+19-CCR5 and

U6+27 CCR5 ribozyme cassettes were prepared using PCR. These ribozymes were prepared from a PCR generated transcriptional template which utilizes the bacteria phage T7 promoter. The transcripts produced mimic exactly (with the exception of the cap on U6+27) those that would be transcribed from the U6 promoter. The in vitro cleavage reactions mediated by these two different RNAs are shown in Figure 3. The U6+19 and U6+27 appended ribozymes cleave the CCR5 target with the same apparent efficiencies.

Two other promoters tested for functional expression of the anti-CCR5 ribozyme were the MoMLV LTR promoter (in an LN retroviral vector) and the adenoviral VA1 promoter (Figs. 6 and 7). The MoMLV promoter construct provides a cap and poly A sequence on the ribozyme transcript and has been used successfully to transcribe anti-tat and tat/rev ribozymes in both cell culture studies and in pre-clinical trials (Zhou, et al., 1994; Bertrand et al, 1997; Bauer et al., 1997). The Adenoviral VA1 promoter, which is a Pol III promoter generates a cytoplasmically localized RNA. Like most Pol III promoters and unlike the U6 promoter, the control regions are internal to the coding sequence. An advantage of this system is that the VA sequences impart a highly stabilized structure which can be very long-lived in the cytoplasm. Shown in Figure 2 is the computer-predicted, thermodynamically most stable structure for the VA1-CCR5 ribozyme. By inserting the ribozyme at the top of the stem loop structure, the ribozyme structure is maintained. The VA1-CCR5 ribozyme construct pictured in Figure 2 has been tested

in vitro for ribozyme cleavage activity. The entire VA1-CCR5 ribozyme RNA was transcribed in vitro using bacteriophage T3 polymerase mediated transcription from a linearized DNA template. It can be seen from the data in Figure 4 that despite being sequestered in VA1 RNA this ribozyme can cleave the CCR5 substrate.

The invention is further illustrated by the following examples, which are not intended to be limiting.

EXAMPLE I

U6+19 CCR5rz and U6+27 CCR5rz in vitro cleavage reactions.

A radiolabelled 103-nucleotide CCR5 target (s) was incubated in the presence of ribozyme at 37°C under the conditions described below (Figure 3). The cleavage reaction products were analyzed on a 6% polyacrylamide, 7M urea denaturing gel. Panel A shows the *in vitro* cleavage reaction of the radiolabelled 103-nucleotide CCR5 substrate (S) by the U6+19 CCR5rz, at 37°C, in presence (+) (lane 2 and 3) or absence (-) (lanes 1 and 4) of 20 mM Magnesium, and at times 5 minutes (lanes 1 and 2) or 1 hour (lanes 3 and 4).

Panel B shows the results of the *in vitro* cleavage reaction of a radiolabelled 103 nucleotide CCR5 substrate (S) by the U6+27 CCR5rz, at 37°C, in presence (lane 2 and 3) or absence (-) (lanes 1 and 4) of 20 mM Magnesium, and at times 5 minutes (lanes 1 and 2) or 1 hour (lanes 3 and 4).

The cleavage products are respectively 72 (CP1) and 31 (CP2) nucleotides. Ribozyme and substrate are respectively at a 5:1 ratio.

EXAMPLE II

VA CCR5rz and VACCR5rzm in vitro cleavage reactions.

A radiolabelled 103 nucleotide CCR5 target (S) was incubated in presence of the V Arz 1 and 2 (different preparations of the same ribozyme construct) (Figure 4, lanes 1-4) or the crippled version, V Arzm1 and 2 (Figure 4, lanes 5-8), in presence (+) or absence (-) of 20 mM MgC12, for 2 hours at 37°C. The cleavage reaction was then analyzed on a 6% polyacrylamide, 7M urea denaturing gel and the results are shown in Figure 4.

The cleavage products are respectively 72 (CP1) and 31 (CP2) nucleotides. Ribozyme and substrate are respectively at a 5:1 ratio.

Lane 9 represents cleavage with the U6+27 CCR5 ribozyme used as a positive control (same reaction as in Fig. 3, panel B, lane 3).

EXAMPLE III

Detection of VA1 and VA1-CCR5rz RNA in transiently transfected cells.

RNA analysis was performed by primer extension on the RNA from transiently transfected 293 cells (Figure 6). The 293 cells were tranfected by either the VA1 plasmid or the VA1-anti-CCR5 plasmid. Two days after the transfection, the RNAs were prepared and used for primer extension with a probe specific to the 3' end of the VA1 RNA.

EXAMPLE IV

Northern blot from 293 transiently transfected cells.

RNA from 293 cells transfected with pVA1 (lane 1), pVA1 anti-CCR5 ribozyme (lanes 42 and 3) and pVA1 anti-

CCR5 mutant (lanes 4 and 5) (Figure 7). The probe used was specific for the 3' end of the VA1 RNA.

EXAMPLE V

Down-regulation of CCR5 receptor in cell culture.

HOS-CD4-CCR5 cells were obtained from the National Institutes of Health, Bethesda, Maryland, U.S.A. These cells were transiently transfected (lipofection) with the various constructs described in Examples I and II. Forty-eight hours after the transfection, a binding assay was performed with the iodinated ligand MIP-1β.

The cells were incubated at 4°C, with 1nM of ^{125}I-MIP-1β for 2 hours, in presence or absence of 100 nM of unlabelled MIP-1β. The cells were then washed 3 times with phosphite-buffered saline and the cell pellets counts were evaluated. The background counts were measured in the presence of the 100 fold excess of cold ligand. The results are shown graphically in Figure 10.

IT IS CLAIMED:

1. A method for downregulating CCR5 in a mammalian cell, comprising administering to the CCR5-producing mammalian cell an effective amount of a ribozyme capable of cleaving RNA coding for said CCR5 receptor.

2. A method of making HIV-resistant cells which comprises transducing said cells with a vector that causes the cells to express an anti-CCR5 ribozyme.

3. A method according to claim 2, wherein the HIV-resistant cells are mononuclear.

4. A method according to claim 2, wherein the transduced cells are hematopoietic precursor cells.

5. A method according to claim 4, wherein the transduced cells are CD34+ human hematopoietic precursor cells.

6. A method according to claim 2, which further comprises transducing said cells with a vector that causes the cells to express one or more ribozymes capable of cleaving a conserved region of the HIV genome.

7. An expression vector containing a nucleic acid encoding an anti-CCR5 ribozyme operatively linked to control signals that direct the expression of such nucleic acid in mammalian cells.

8. An expression vector according to claim 7 wherein the vector is U6+19.

9. An expression vector according to claim 7 wherein the vector is U6+27.

10. An expression vector according to claim 7 wherein a vector is an LN retroviral vector containing the MoMLV LTR promoter.

11. An expression vector according to claim 7 wherein the vector contains the adenoviral VA1 promoter.

12. A method for the treatment as prophylaxis of HIV infection in a subject, which comprises administering to said subject a therapeutically or prophylactically effective amount of an anti-CCR5 ribozyme.

13. The method of claim 12, wherein said anti-CCR5 ribozyme is administered by in vivo expression of a nucleic acid encoding said anti-CCR5 ribozyme to produce a therapeutically or prophylactically effective amount of said ribozyme in said subject's body.

14. The method of claim 13, wherein said in vivo expression is accomplished by transducing cells ex vivo with a nucleic acid encoding said anti-CCR5 ribozyme operatively linked to control signals that direct the expression of said nucleic acid in said cells and introducing said transduced cells into said subject in an amount sufficient to result in the production of a therapeutically or prophylactically effective amount of said ribozyme in said subject's body.

15. The method of claim 13, wherein a vector containing a nucleic acid encoding said anti-CCR5 ribozyme is administered to said patient in an amount sufficient to transduce cells in vivo and direct the expression of a therapeutically or prophylactically effective amount of said ribozyme in vivo.

16. The method of claim 12, wherein said anti-CCR5 ribozyme is administered in liposomes in a therapeutically or prophylactically effective amount.

17. A method of treating HIV infection comprising administering to a patient an anti-CCR5 ribozyme in combination with one or more ribozymes targeted to conserved sequences in HIV.

18. A CD34+ human hematopoietic precursor cell transduced with a vector encoding a ribozyme capable of cleaving CCR5 RNA.

19. A CD34+ human hematopoietic precursor cell of claim 18 further comprising a ribozyme targeted to conserved sequences in HIV.

20. A CD34+ human hematopoietic precursor cell transduced with a vector of claim 8, claim 9, claim 10, or claim 11.

21. A ribozyme targeted to the second GUC of the CCR5 mRNA.

22. A ribozyme construct having the ribonucleotide sequence:
3'CACAGUUCAAAGCAGGUGUGCCUGAGUAGUCGUUAGAUA5'.

23. The ribozyme of claim 22, wherein one or more of the ribonucleotides outside of the catalytic region are replaced with deoxyribonucleotides.

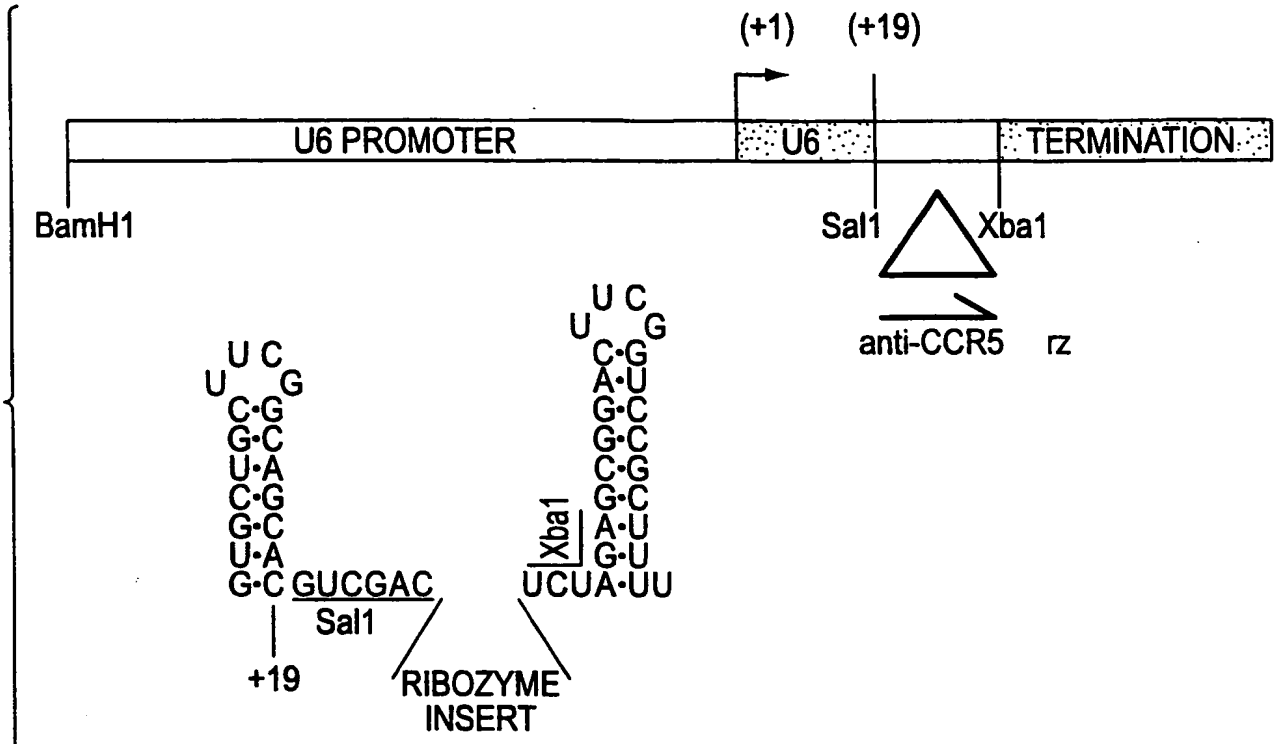

FIG. 1A

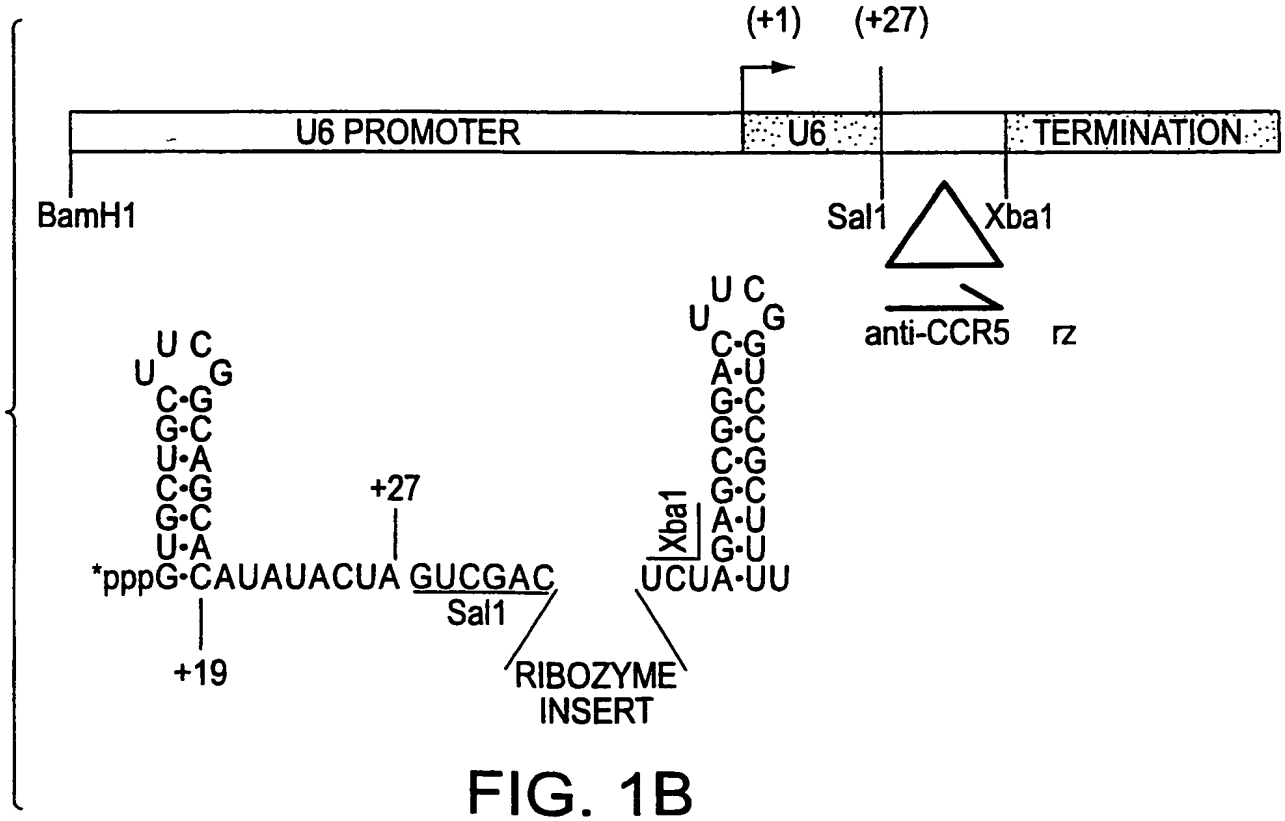

FIG. 1B

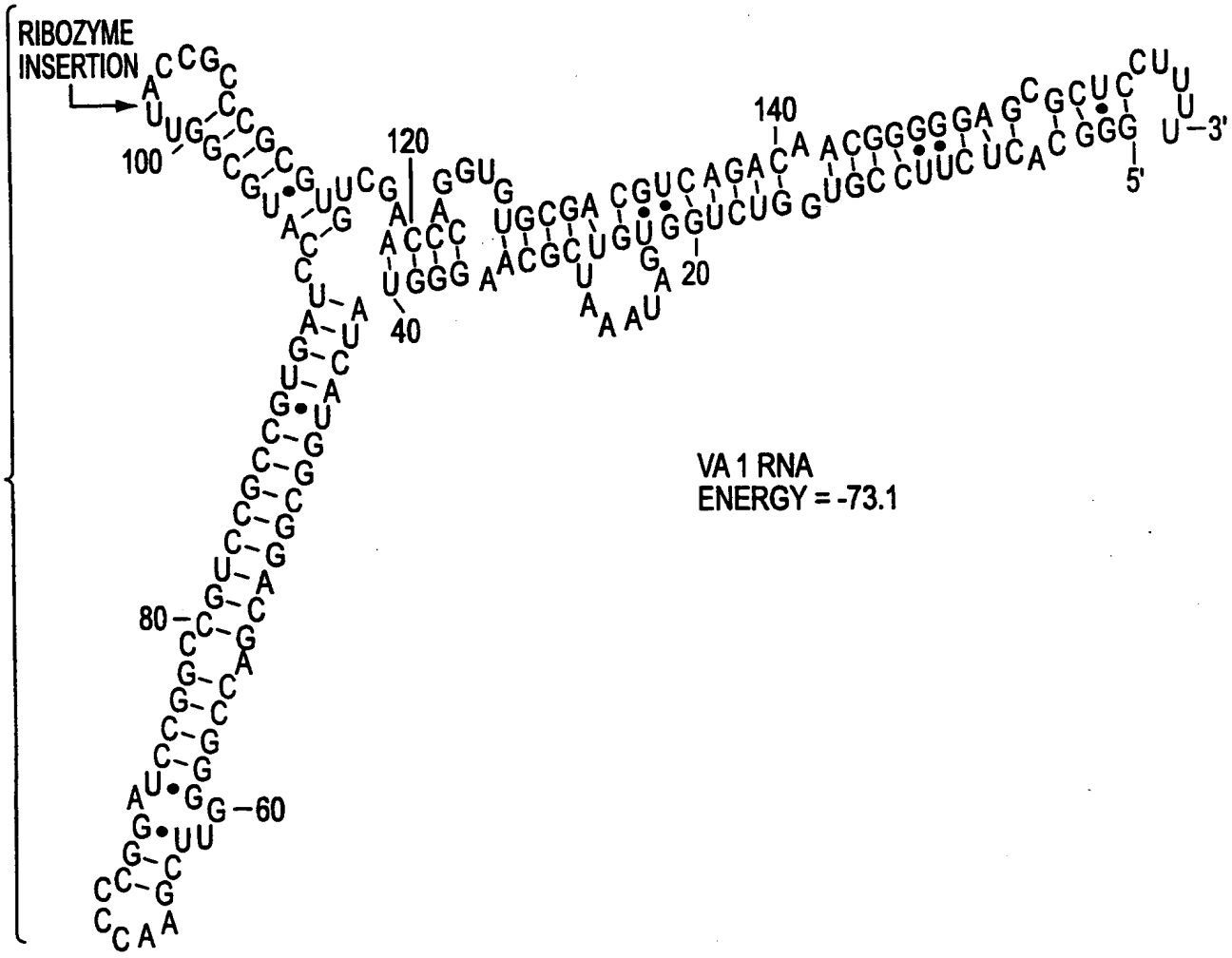

FIG. 2A

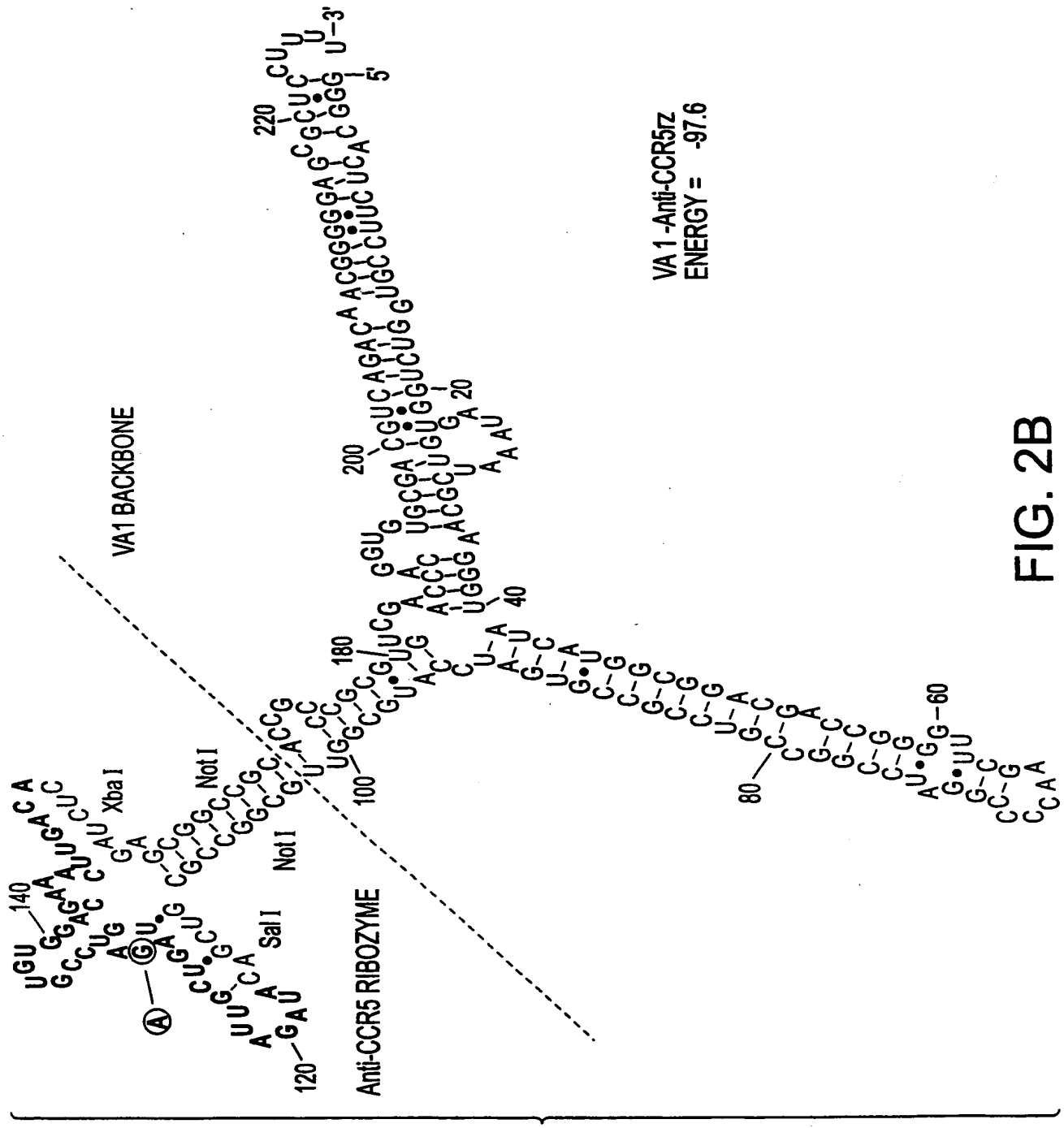

FIG. 2B

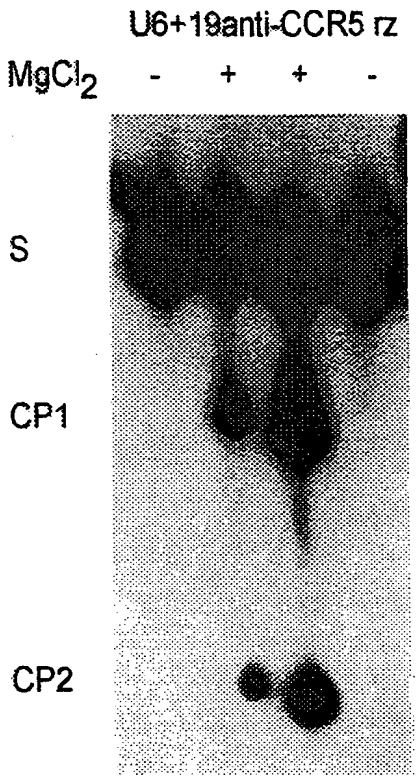

FIG. 3A

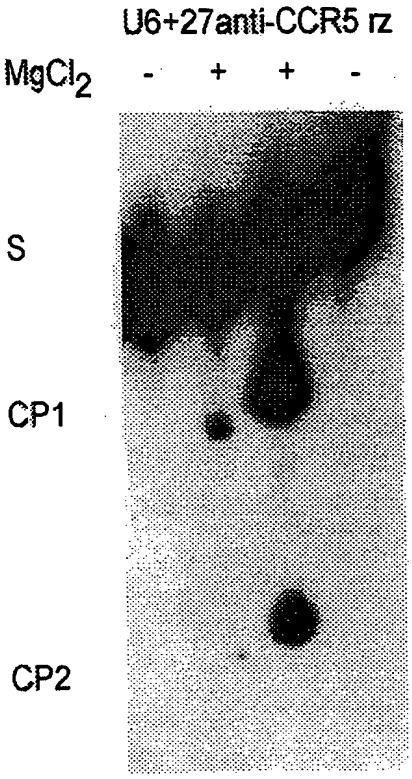

FIG. 3B

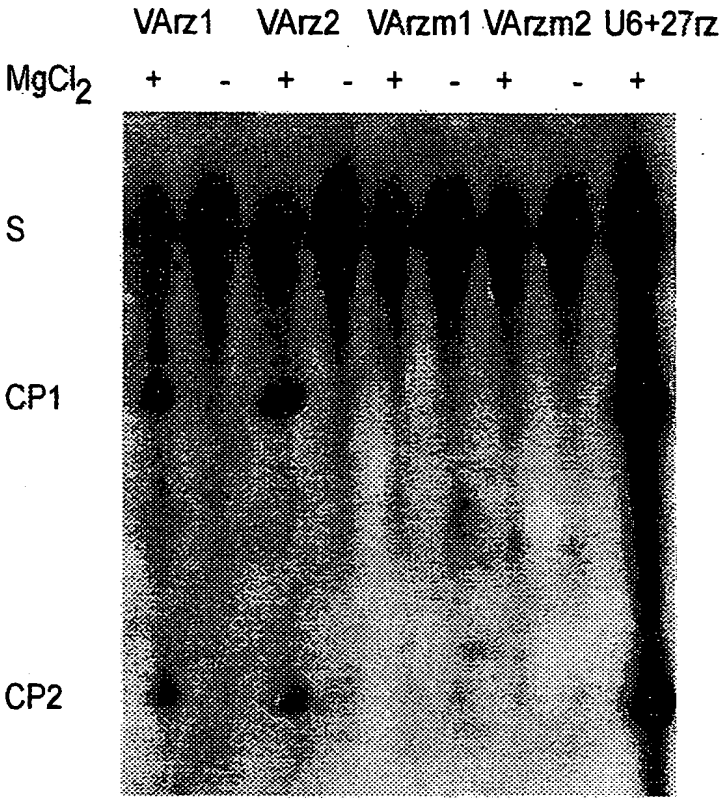

FIG. 4

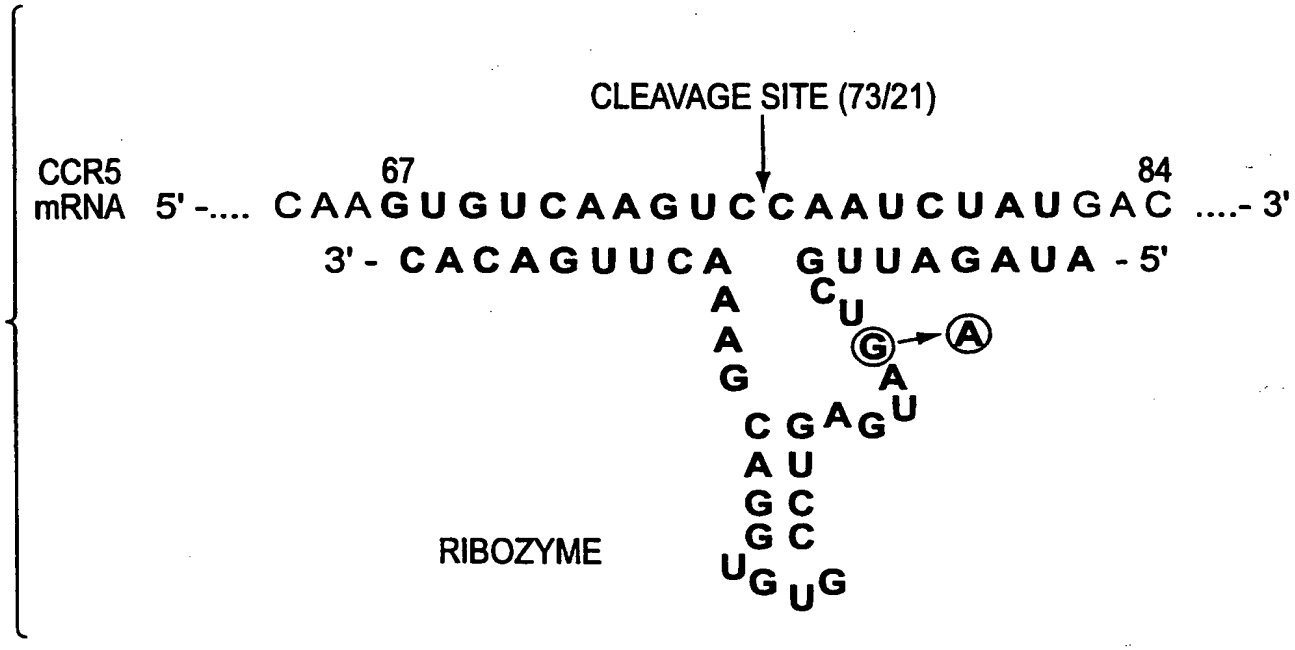

FIG. 5

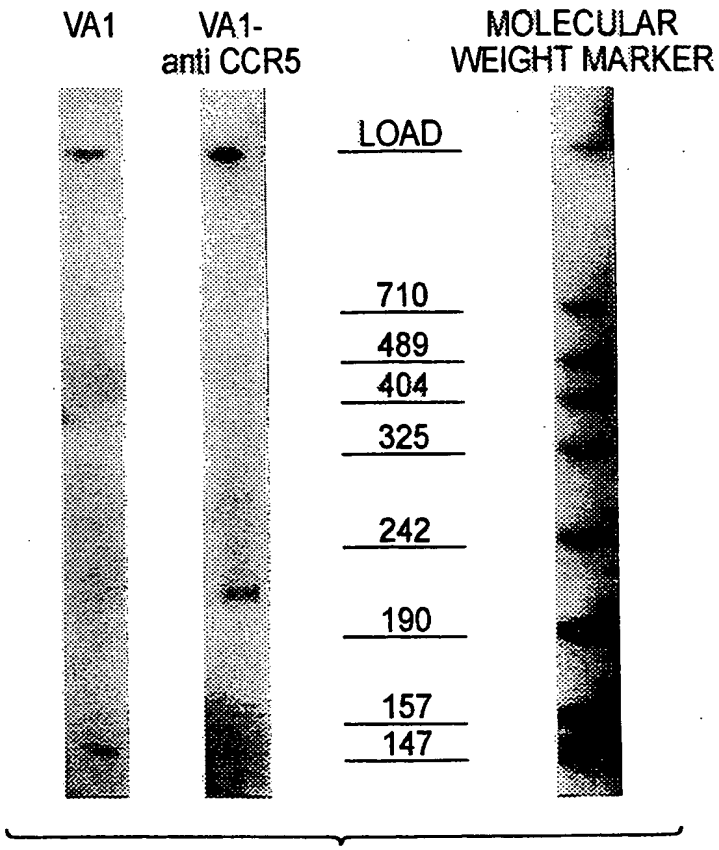

FIG. 6

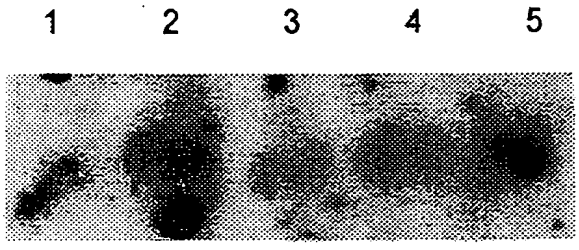

FIG. 7

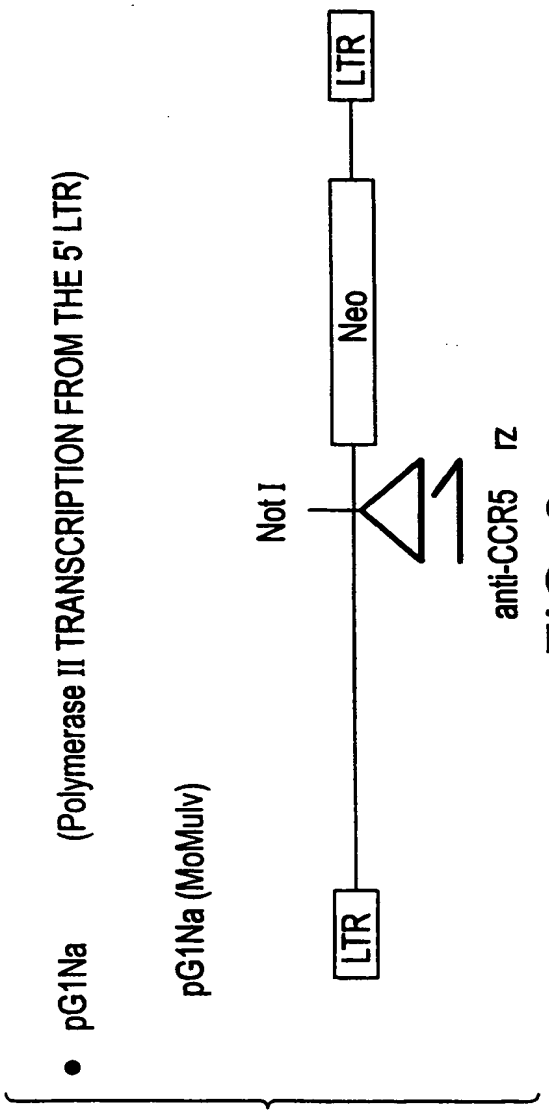

FIG. 8

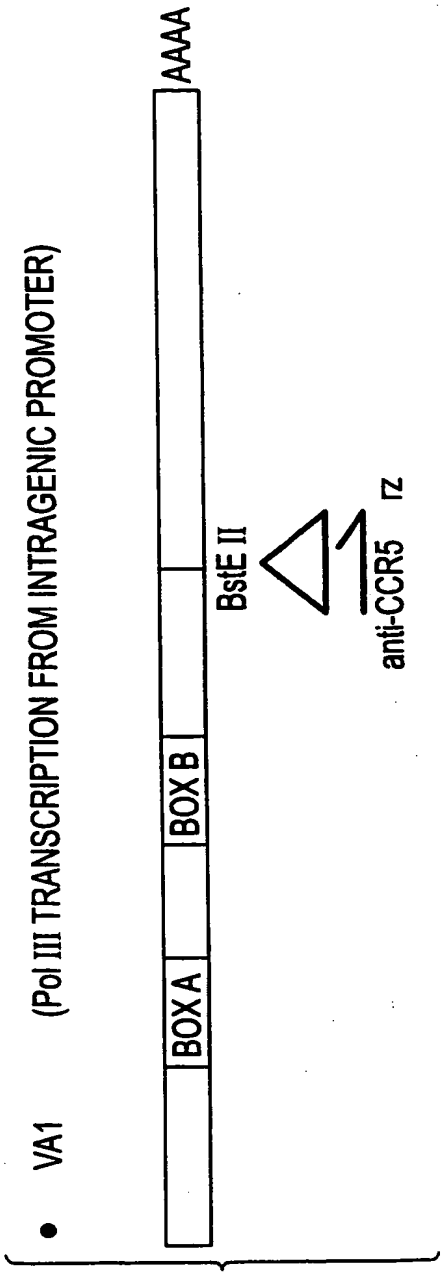

FIG. 9

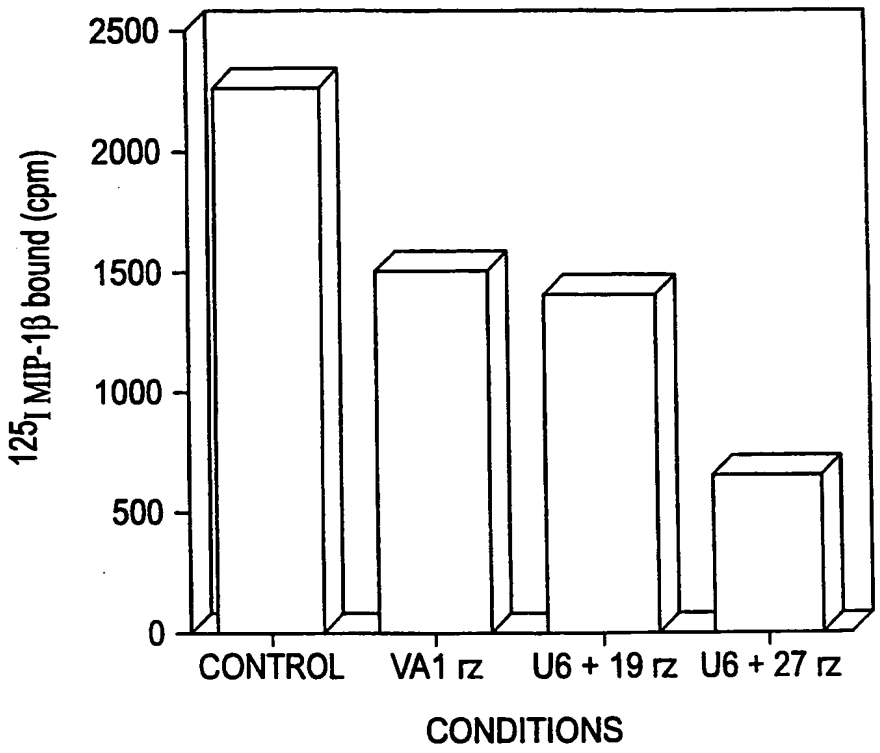

FIG. 10

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Rossi, John J.
Cagnon, Laurence

(ii) TITLE OF THE INVENTION: Ribozymes

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Rothwell, Figg, Ernst, and Kurz
(B) STREET: 555 Thirteenth Street, N.W. Suite 701 East
(C) CITY: Washington
(D) STATE: DC
(E) COUNTRY: USA
(F) ZIP: 20004

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Figg, E. A
(B) REGISTRATION NUMBER: 27195
(C) REFERENCE/DOCKET NUMBER: 1954-206

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 202-783-6040
(B) TELEFAX: 202-783-6031
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: rRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CACAGUUCAA AGCAGGUGUG CCUGAGUAGU CGUUAGAUA 39

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

UAUCUAACCU GAACUGUG 18

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C12N 15/11, 9/00, A61K 31/70, C12N 15/86, 5/10 // C07K 14/715

A3

(11) International Publication Number: **WO 99/46372**

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(30) Priority Data:
09/038,741 11 March 1998 (11.03.98) US

(71) Applicant: CITY OF HOPE [US/US]; 1500 East Duarte Road, Duarte, CA 91010-3000 (US).

(72) Inventors: ROSSI, John, J.; 6255 Terracina Avenue, Alta Loma, CA 91737 (US). CAGNON, Laurence; Apartment 23, 1817 2nd Street, Duarte, CA 91010 (US).

(74) Agents: FIGG, E., Anthony et al.; Rothwell, Figg, Ernst & Kurz, Suite 701 East, Columbia Square, 555 13th Street N.W., Washington, DC 20004 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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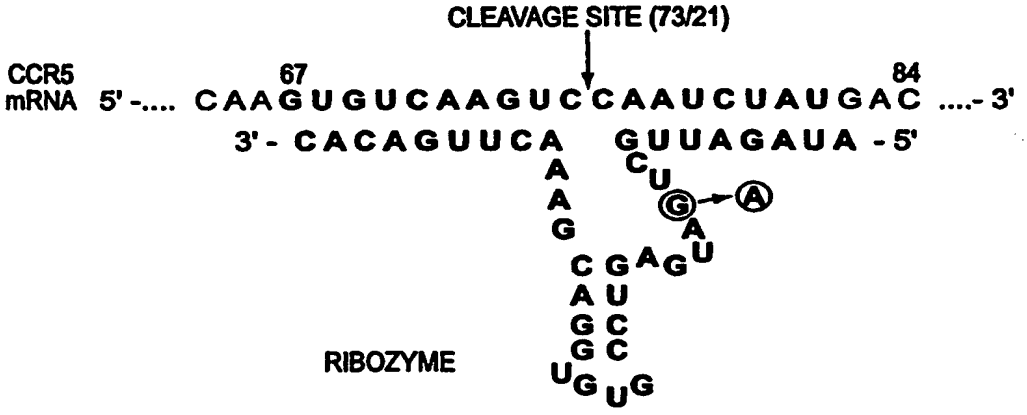
(88) Date of publication of the international search report:
4 November 1999 (04.11.99)

(54) Title: RIBOZYMES CAPABLE OF INHIBITING THE EXPRESSION OF THE CCR5 RECEPTOR



(57) Abstract

This invention provides ribozymes and combinations thereof, to cleave RNA sequences. The invention also provides a method of treating HIV-1 by down-regulating the CCR5 receptor.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/05011

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 C12N9/00 A61K31/70 C12N15/86 C12N5/10
//C07K14/715

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 05798 A (AARON DIAMOND AIDS RESEARCH CE) 12 February 1998 (1998-02-12) page 11, line 17 -page 12, line 2 page 18, line 11 - line 18	1-3,7, 12-15
Y	page 57 -page 59	2-20
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
2 September 1999	14/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Andres, S

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INTERNATIONAL SEARCH REPORT

National Application No
PCT/US 99/05011

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	BAUER, G. ET AL.: "Inhibition of human immunodeficiency virus-1 (HIV-1) replication after transduction of granulocyte colony stimulating factor-mobilized CD34+ cells from HIV-1 infected donors using retroviral vectors containing anti-HIV-1 genes." BLOOD, vol. 89, 1 April 1997 (1997-04-01), pages 2259-2267, XP002114033 ISSN: 0006-4971 cited in the application the whole document ---	2-6,10, 12-15, 17-20
Y	BERTRAND E ET AL: "THE EXPRESSION CASSETTE DETERMINES THE FUNCTIONAL ACTIVITY OF RIBOZYMES IN MAMMALIAN CELLS BY CONTROLLING THEIR INTRACELLULAR LOCALIZATION" RNA, vol. 3, no. 1, 1997, pages 75-88, XP000646611 ISSN: 1355-8382 cited in the application the whole document ---	7-11
Y	TAYLOR N R ET AL: "CHIMERIC DNA-RNA HAMMERHEAD RIBOZYMES HAVE ENHANCED IN VITRO CATALYTIC EFFICIENCY AND INCREASED STABILITY IN VIVO" NUCLEIC ACIDS RESEARCH, vol. 20, no. 17, 11 September 1992 (1992-09-11), pages 4559-4565, XP000644533 ISSN: 0305-1048 cited in the application	12,16
A	the whole document ---	23
X	WO 97 45543 A (COMBADIERE CHRISTOPHE ;FENG YU (US); US HEALTH (US); ALKHATIB GHAL) 4 December 1997 (1997-12-04) page 34, line 15 -page 43 claims 59-68 ---	1-3, 12-16
A	GOOD P D ET AL: "EXPRESSION OF SMALL, THERAPEUTIC RNAS IN HUMAN CELL NUCLEI" GENE THERAPY, vol. 4, no. 1, 1997, pages 45-54, XP000646610 ISSN: 0969-7128 cited in the application the whole document ---	7-9

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INTERNATIONAL SEARCH REPORT

National Application No
PCT/US 99/05011

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROSSI J J ET AL: "RIBOZYMES AS ANTI-HIV-1 THERAPEUTIC AGENTS: PRINCIPLES, APPLICATIONS, AND PROBLEMS" AIDS RESEARCH AND HUMAN RETROVIRUSES, vol. 8, no. 2, 1 February 1992 (1992-02-01), pages 183-189, XP002026934 ISSN: 0889-2229 ---	
P,X	GONZÀLEZ, M. ET AL.: "A hammerhead ribozyme targeted to the human chemokine receptor CCR5" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS., vol. 251, 20 October 1998 (1998-10-20), pages 592-596, XP002103073 ISSN: 0006-291X the whole document ---	1-3,6,7, 12-15, 17,21-23
P,X	WO 98 17308 A (LEAVITT MARKLEY C ;BARBER JACK (US); FENG YU (US); IMMUSOL INC (US) 30 April 1998 (1998-04-30) page 4 -page 7 page 14, line 26 -page 26, line 24 example 2 claims ---	1-7, 10-21,23
E	WO 99 36518 A (EAGLES PETER ANTHONY MINTER ;ZHENG RICHARD QIHAO (GB); BTG INT LTD) 22 July 1999 (1999-07-22) the whole document -----	1-3,7, 12-16, 21,22

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/05011

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-6 (as far as in vivo methods are concerned and claims 12-17 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

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Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
PCT/US 99/05011

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9805798 A	12-02-1998	AU 4055897 A	25-02-1998
WO 9745543 A	04-12-1997	AU 3375697 A	05-01-1998
WO 9817308 A	30-04-1998	AU 5101398 A	15-05-1998
WO 9936518 A	22-07-1999	NONE	